AN ANALYSIS OF THE SPAWNING HABITS AND SPAWNING STIMULI OF CHÆTOPLEURA APICULATA (SAY).

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The phenomenon of periodicity in the spawning activities of animals has attracted wide attention since its most striking example was made known in a series of papers by Collin, Kraemer, Friedlander, Izuka, and Mayer ('97–'08). These papers give the results of careful studies of the spawning habits of the Palolo Worm and show that both the Atlantic and Pacific species shed their gametes during particular phases of the moon and tides. The time of their swarming is so definite that it may be predicted as certainly as an eclipse.

Since these papers were published a distinct periodicity in the time of spawning has been revealed for a number of animals, related not merely to season, but to the time of the month. Many animals have long been known to spawn regularly at a certain time of the day or night. This paper does not deal with the problem of such diurnal periodicity, but only with lunar periodicity. Most of the animals which are known to exhibit a periodicity of the latter category are annelids, and the degree of perfection of their periodicity varies with that which is just discernible, as in *Nereis dumerilii,* to that of the Atlantic palolo, *Eunice fucata,* in which the swarming is restricted to a certain phase of the moon once a year. This curious phenomenon is not restricted to animals, but is shown quite as perfectly by the brown alga, *Dictyota dichotoma,* as described by Williams ('05), Hoyt ('07), and Lewis ('10). Some other species of the Dictyotaceæ also show a similar periodicity in the time of freeing their gametes.

PERIODICITY IN CHÆTOPLEURA.

When an attempt was made, in the summer of 1919, to obtain the eggs of *Chætopleura apiculata* for embryological study, it was

discovered that they could not be had at all times. They were obtained plentifully from July 12 to 20 and then spawning suddenly ceased. The writer naturally supposed that the end of the spawning season had been reached and that no more eggs could be expected that summer. However, experiments were continued at intervals of a few days, and at the approach of the next full moon eggs were again obtained. This time the period of spawning extended from August 8 to 11, inclusive. No eggs were obtained after the eleventh of August, although numerous attempts to induce further spawning were made. By referring to the table which follows, it will be seen that spawning began at the approach of full moon in July and continued thereafter for approximately one week (or until the third quarter), then ceased for a time to begin again in like manner at the time of the next full moon, thus indicating a distinct lunar periodicity.

TABLE.

Phase of moon.	Dates on which spawning occurred, summer of 1919.
Full moon.....	July 12.
	July 13.
	July 14.
	July 16.
Third quarter.....	July 20. Spawning active, apparently at maximum, many eggs laid by large proportion of females. Spawning began at 8.00 P.M. and continued until 2.00 A.M.
	August 8. Spawning occurred about 9.00 P.M. No eggs had been obtained during the preceding two weeks which included the new moon and first quarter.
Full moon.....	August 10.
	August 11.
	No eggs obtained after August 11, although several trials were made.

During the summers of 1920 and 1921 experiments were continued for the purpose of determining the limits of the spawning season and the relation of spawning activity to the cycles of the moon. As a result of these experiments the time limits of the spawning periods were found to be much less restricted than the data collected in 1919 indicated. In general, the data obtained during the last two years show that eggs may be had in small quantities at almost any time. By far the greatest spawning activity, however, comes at the approach of full moon and continues for ten

days or two weeks. This more active spawning period includes two or three days preceding full moon and continues until after the third quarter, or with slight abatement until two or three days after new moon. In 1921 the diminution of egg-laying came promptly at new moon. The periods of excessive spawning activity are followed by periods of low activity during which spawning is practically suppressed. These periods of low spawning activity extend roughly from new moon until the approach of full moon.

More specifically stated, there were ten-day periods in each of the months of June, July, and August, during which spawning activity was remarkable for the abundance of eggs extruded, and there were other periods between each of these high points when spawning activity was diminished.

The low spawning periods were somewhat more distinct in 1921 than in 1920. In both years approximately sixty per cent. of the eggs obtained were spawned between full moon and the third quarter, and perhaps seventy-five per cent. during the periods beginning three days before full moon and extending to the next new moon.

The data obtained during the three years agree except that the spawning season of the last two years extended over longer periods, and that the lunar periodicity was less marked than in 1919, when spawning was confined to a few days in each month.

A further comparison of the results obtained during the three years is interesting in that the total number of eggs secured during 1920 was almost double that of 1921 and very far in excess of that of 1919. There is no apparent reason for this variation in egg production from year to year, but similar observations on other species of animals are common.

It may not be amiss in this connection to point out that Lillie and Just ('13) have found spawning activities in *Nereis limbata* which are quite similar to those of *Chætopleura,* but the periodicity of the two species may or may not be identical.

In what is to follow reasons are given for believing that the spawning periods of *Chætopleura,* under natural conditions, are more restricted than the results of the experiments carried out under laboratory conditions indicate.

Spawning under Laboratory Conditions.

It may be that the spawning habit and presumably other behavior of animals under laboratory conditions is not a reliable criterion for normal behavior in nature. This comes out most clearly when all of the factors which influence spawning under artificial conditions are considered.

It may almost be stated as a general rule that sexually mature marine animals are likely to shed their ripe eggs when placed under abnormal conditions. It would seem, therefore, to be a futile, or at least an unnecessary procedure, to attempt to duplicate normal conditions in the laboratory as a means of inducing spawning. For instance, in order to secure the eggs of the small lamellibranch, *Cumingia,* it is only necessary to take them from the sand in which they live and expose them to the air for an hour or two, whereupon, when placed in a pan of quiet sea-water, they begin to spawn abundantly within half an hour. Eggs may be obtained in this manner at almost any time during the summer, and it is not uncommon for every female which is brought into the laboratory to spawn. It is not probable that many of these females would have spawned at these particular times if they had been left undisturbed in their normal situations.

Consider the case of the annelid, *Hydroides.* In order to cause them to spawn it is sufficient to break away their calcareous tubes and place them in sea water. Under these conditions they shed their sexual products within a few seconds. Dr. E. E. Just states that it is only necessary to pinch their tentacles or snip them off with scissors to induce spawning. They need not be removed from their tubes. It would appear, therefore, that the spawning of mature sexual products, in these cases, is influenced more by shock than by normal conditions. In fact, the more unnatural the conditions are made the better. However, after the shock has been applied, they require to be placed in a quiet situation, and presumably the more nearly it approaches normal conditions the better.

If these data have general significance, we may suppose that experiments which are performed in laboratories upon the behavior of animals, especially with reference to spawning, should be

checked up as far as possible by observations of their habits under natural surroundings where the possibility of shock is eliminated.

The writer is presuming, therefore, that the spawning periods of *Chætopleura* are restricted to ten days in each of the summer months, extending approximately from full moon to the third quarter. This, however, is not set forth as a fact. If laboratory data are wholly trustworthy, the spawning periods extend from full moon to new moon with the qualifications noted in the text.

Conditions Affecting Spawning.

As a matter of convenience in the study of the spawning periods of *Chætopleura,* it was found most advantageous to remove the animals from the shells to which they normally cling and to keep them in shallow crystallization dishes.

It was soon discovered that they spawn only at night, usually beginning about 7.30 or 8 P.M. and continuing until 10.30 P.M. It is not uncommon for them to spawn at intervals throughout the night.

During the day the animals were kept aërated by means of running water, but toward evening they were washed free from sediment and placed in dishes on laboratory tables. They rarely spawned in running water, but the change to quiet water furnishes an effective stimulus to spawning. Under these conditions one or more males usually respond within fifteen minutes, females somewhat later. If allowed to remain in running water, neither males nor females respond, so far as has been observed. It is therefore evident that these animals are affected by currents of water, and one naturally suspects that they spawn under natural conditions, either at low or high tide when the water is slack. No convincing evidence that they spawn at the time of low or high tide was obtained from laboratory experiments.

Although these animals rarely spawn in water that is strongly agitated, they spawn readily in dishes which constantly receive a fresh supply of sea-water, provided the inflowing stream does not perceptibly disturb or agitate the water in the dish. This shows that spawning is not induced by the accumulation of carbon dioxide. The same conclusion may be drawn from the fact that spawning

frequently begins within a few minutes after the animals have been placed in quiet water, and that it is practically over before the surrounding water is greatly affected by respiration. During the summer of 1920, at the height of the breeding season, a few females spawned in strongly agitated water. This occurred only once (July 8).

The number of eggs obtained from one female varies from about three hundred to three thousand, the average number being about sixteen hundred. As a rule only a few eggs are obtained during the first night after the animals have been collected. The greater number of females spawn the second night. A few spawn also during the third and fourth nights, but usually not to any considerable extent.

Spawning Stimuli.

Soon after being placed in quiet water the males begin to emit sperms in a double stream which soon become dispersed as clouds throughout the containing dish. The females respond more slowly after from one to three hours. One is therefore likely to suppose that something is expelled with the sperm which serves to stimulate the females to extrude their eggs.

This phenomenon was noted by M. M. Metcalf ('92) while studying two species of *Chiton* in aquaria. It was also described by Harold Heath ('05). F. R. Lillie and E. E. Just ('13) showed, without question, that in the case of *Nereis limbata* a substance is expelled with the sperm which is an effective stimulus to the female to shed her eggs promptly. In similar manner they found that the males will not shed their sperm except in the presence of a female which contains mature eggs or when placed in a dish of sea-water in which a mature female had been allowed to remain for an hour or more.

In order to test the assumption that this is also the case in *Chætopleura,* twenty mature individuals were washed thoroughly with fresh water and isolated in finger bowls of sea-water. During the night three of these females spawned and six males shed sperms in quantity, thus showing that neither males nor females derive any stimulus essential for spawning from the opposite sex. It is evident that the females were not induced to spawn by the

presence of the spermatic fluid from the fact that none of the eggs initiated development. After some hours had elapsed these eggs were artificially fertilized and a considerable percentage of them went through cleavage stages, showing that they were normal, mature eggs, and that they had not previously been subjected to spermatozoa.

This experiment, first performed in 1920, was verified during the past summer.

Both sexes are apparently affected by the change from running to quiet water (tide changes), presumably also by the change in pressure between high and low tides (mechanical shock), and most certainly by moonlight.

It is evident that these factors have not been analyzed satisfactorily, but Mayer ('07 and '08), by actual controlled experiments, has contributed at least a first step toward a solution. The writer is of the impression that Scott's ('09) explanation, in the case of *Amphitrite,* that the periodic maturing of the sexual products is due to the warming effect of the sun at periods of especially low tides, can not be generally applied and is not applicable to *Chætopleura.*

The non-committal statement made by Lillie and Just ('13), that the maturing of the sexual elements of these animals is dependent upon phases of the moon, involving, through lunar tidal variations, rhythmical alterations of conditions of nutrition, is less open to criticism and is as exact as the known facts warrant at the present time.

Many interesting suggestions concerning the cause of periodicity in spawning have been made by various authors, but most of them are undoubtedly wide of the mark.

Friedlander ('98–'01), after reviewing various possibilities, finally admits that he is wholly in the dark and arrives at the conclusion that no theories yet proposed are adequate to explain periodicity in spawning.

Mayer ('07 and '08) states, upon the authority of experimental data, that moonlight is the effective cause, and that the tides are unnecessary but contributing causes.

Hemplemann ('11) believes that the moonlight, acting through a period of several days, is the stimulus to the maturing of the

sexual products, and that it may also be the immediate cause of swarming.

A theory proposed by Brunelli and Schroener,[1] that the swarming has its origin in original accidental shocks to the nervous system of these worms, and that their swarming activities finally became spontaneous or hereditary in a form that was favorable to the propagation of the species, is probably true, but it explains nothing. That the habit of spawning periodically has become hereditary is shown by the fact that the animals under discussion will spawn at the proper time even when the normal stimuli are altered or absent. See Mayer ('07).

Most of these theories are based upon abstract reasoning and not upon experimental data. It is apparent that there is something yet to be done before we can understand this curious phenomenon and its relation to the moon. That this habit has become more or less hereditary is shown by several authors. However, it must have originated in relation to external stimuli which are felt periodically. The cause of this type of periodicity must be fundamentally the same in all cases, although it is exhibited in a variety of ways, and at different times relative to the phases of the moon.

Experiments on Chætopleura.

Summer, 1920.

I.

June 20–23:

20 Chitons collected June 20.

1st night, no eggs.

2d night, no eggs.

3d night, no eggs.

* First Quarter, June 23.

II.

June 24–27:

30 Chitons collected June 24.

1st night, no eggs, some spermatozoa.

2d night, no eggs.

3d night, no eggs.

* It is due to the last two authors mentioned to state that the writer has read only quotations from their papers.

III.

June 27–30:

15 Chitons collected June 27.

1st night, four females laid eggs; spermatozoa abundant.

2d night, two or three females spawned lightly.

3d night, two or three females spawned lightly.

IV.

June 28–30:

100 Chitons collected June 28.

1st night, no eggs.

2d night, great quantities of eggs from 12 to 15 females; spermatozoa abundant; eggs laid 9–12 P.M.

3d night, two females spawned in quantity; a few eggs from several others.

4th night, two specimens spawned.

5th night, a few still spawned.

Note.—Nearly all spawned second night. Spawning is nearly always on 1st or 2d night after collecting. Spermatozoa appear first in every case observed.

V.

*Full moon, July 1.

July 2–3:

20 Chitons collected July 2.

1st night, two spawned, one of these about 10 P.M.

2d night, two spawned; spermatozoa shed in abundance as soon as animals were placed in quiet water.

VI.

July 3–4:

158 Chitons collected July 3. Placed 25 per dish.

1st night, two spawned.

2d night, about 20 females spawned, and about 30 or more males shed spermatozoa. (This group continued to spawn freely for five nights.)

VII.

July 6–8:

100 Chitons collected July 6.

1st night, several specimens spawned about 10 P.M., and a few more after 1 A.M.

2d day, several females spawned between 9 and 10 A.M., July 8, and one or two continued at intervals during the afternoon; spermatozoa also abundant. Beginning about 5 P.M., many females (35–40) spawned in great abundance, laying 2,000–2,500 eggs each.

Note.—July 8 seemed to be the height of the spawning season. Spawning occurred day and night, practically all night and more or less during the entire day. Spawning during the day not seen before.

* Last Quarter, July 8.

Note.—Experiments so far show that females spawn at the approach of full moon, a few days before. Spermatozoa are shed for longer periods and more readily than the eggs. Spermatozoa appear in clouds very soon after animals are placed in quite water, 5–10 minutes, and sometimes continue nearly all night. Females sometimes spawn the first night after collecting, but more abundantly the second night.

VIII.

July 11–14:

100 specimens collected July 11.

1st night, several specimens spawned.

2d night, no eggs.

3d night, several specimens spawned.

4th night, several specimens spawned.

Note.—This group was not well cared for, because attention was necessarily diverted to other matters. There was considerable if not abundant spawning, although not comparable to that of July 8.

* New moon, July 15.

IX.

July 17–18:

100 specimens collected July 17.

1st night, two specimens spawned.

2d night, several specimens spawned in quantity, 7–10 P.M.

* First Quarter, July 22.

X.

July 22–25:

100 specimens collected July 22.

1st night, three spawned about one half usual amount of eggs.

2d night, about six specimens spawned in quantity, one half to two thirds maximum.

3d night, two specimens spawned lightly and a few eggs from others.

4th night, none spawned.

XI.

July 28–30:

75 specimens collected July 28.

1st night, one spawned; a few eggs from a second.

2d night, about ten specimens spawned in great abundance, some about three thousand eggs.

* Full moon, July 30.

XII.

August 4–6:

75 specimens collected August 4.

1st night, very little spawning.

2d night, about eight spawned in abundance.

XIII.

* Last Quarter, August 7.

August 7, experiment worthless. Chitons suffocated by standing without water.

XIV.

August 10–11:

120 specimens collected August 10. Good lot.

1st night, no eggs.

2d night, three specimens spawned 8–10 P.M.; almost none after 10 P.M.

* New moon, August 13.

XV.

August 20–22:

112 specimens collected August 20.

1st night, no eggs and no spermatozoa.

2d night, one female spawned lightly (300 eggs), also twenty eggs from another, and nine from a third.

3d night, one female spawned average number of eggs (1,500), two others about 300 each.

XVI.

August 24–26:

94 specimens collected August 24.

1st night, no eggs, no spermatozoa.

2d night, two males emitted clouds of spermatozoa; one female spawned good bunch of eggs, a second thirty-two eggs.

3d night, three females spawned lightly (100 to 300 eggs each).

Remark.—Very light spawning since new moon.

* Full moon, August 29.

XVII.

August 28–31:

30 Chitons collected August 28. Left in supply department in insufficient amount of water until 10 P.M. (Fault of collectors.)

1st night, experiment set at 10 P.M.; no eggs and no spermatozoa.

2d night, two females spawned lightly and one male emitted spermatozoa.

3d night, two females spawned maximum quantity of eggs, 2,500 each.

4th night, no eggs.

Note.—The last experiment is more or less untrustworthy, because the chitons were partially suffocated by improper treatment after collecting. The group, however, shows a distinct revival of spawning in that thirty chitons spawned many times more eggs than the larger group of the preceding experiments.

Experiments, 1921.

Experiments on Chætopleura. Summer of 1921.

* Full moon, June 20.

I.

Experiment 1, June 24–26.

1st night, of twenty-five large chitons collected June 24, three and possibly four spawned in fair quantity, first night, perhaps 50–60 per cent. of maximum; none spawned before 10 P.M.

2d night, spawning began at 10.30 P.M.; probably over before midnight, as indicated by development of embryos next morning; five females out of twenty-five animals spawned one half to two thirds maximum; good quantity.

Remark.—Experience of last year has shown that most of the spawning is done the second night after collecting. Therefore, it is best to consider only 2d night in studying spawning habits, or else compare experiments as a whole.

* Last Quarter, June 28.

II.

Experiment 2, June 30–July 2.

30 specimens collected June 30.

1st night, no females spawned; a few males shed spermatozoa (2 or 3).

2d night, three females spawned about one half maximum.

3d night, two females spawned in considerable quantity, a third one lightly.

Remark.—Impression of good spawning activity during the last week in June. Plenty of eggs for embryological study. Isolated specimens also spawned readily and in quantity.

III.

Experiment 3, July 2–5.

60 specimens collected Saturday, July 2; 30 placed in running water and 30 placed, as usual, on laboratory tables.

1st night, none spawned in either case; one male was shedding spermatozoa at 7.30 A.M. Sunday morning.

2d night, of those placed in running water one spawned one fourth maximum; of those placed on laboratory table two spawned lightly; several males shed spermatozoa in quiet water (abundant from 8 to 11 P.M.); very little in running water.

3d night, those placed on laboratory table spawned about 25 eggs; those placed in running water spawned 12 eggs; one specimen spawned in each case; males shed spermatozoa in considerable quantity in quiet water.

Remark.—Very light spawning from this group.

* New moon, July 5.

IV.

Experiment 4, July 5–7.

40 Chitons collected July 5. Placed in running water promptly, but not set for experiment night of July 5.

2d night, set in two lots, one in running water and one on laboratory table in quiet water; twenty good specimens each; of those on laboratory table one spawned 100 eggs more or less; of those in running water two spawned not to exceed 100 eggs each, between 9–11 P.M.

3d night, set as above; none spawned during the night, males nor females.

Remark.—Almost no spawning from this group.

V.

Experiment 5, July 7–9.

80 Chitons collected in afternoon, July 7. Divided into two lots, 40 each. Medium size to large specimens. Good lot. One group placed in running water and one in quiet water on laboratory table, as usual, about 7 P.M.

1st night, none spawned in either dish, males nor females.

2d night, eggs laid by two females in quiet water, about 100 eggs each or one tenth maximum; spawned at 11 P.M.; none laid by those in running water; one male shed great cloud of spermatozoa in quiet water.

3d night, no eggs and no spermatozoa obtained in either dish.

Remark.—Spawning at this time and for several days past practically suppressed.

* First Quarter, July 11.

VI.

Experiment 6, July 12–14.

60 Chitons collected in afternoon, Tuesday, July 12, and kept in running water at supply department night July 12.

2d night, July 13, experiment set in two dishes as usual; of those in running water none spawned; of those in quiet water on laboratory table three males shed spermatozoa freely before 8 P.M., and others during the night; one female spawned lightly one fourth the maximum amount about 10 P.M.; about thirty-five additional eggs were spawned by one female during the night.

3d night, at 10.30 P.M. a few males shed spermatozoa abundantly; one female spawned and only 40–50 eggs in quiet water; one female in running water spawned about 30 eggs during the night.

Remark.—Spawning practically suppressed.

VII.

Experiment 7, July 14–16.

90 Chitons collected afternoon of Thursday, July 14. Placed about 6 P.M. in three dishes. One left in running water. Two placed in quiet water on laboratory table.

1st night, spermatozoa in small amount shed by three males before 10 P.M. in quiet water; no eggs in either dish up to 11 P.M.; about 40 eggs obtained from one female in quiet water during the night.

2d night, experiment set as usual 6 P.M.; those in quiet water began to shed spermatozoa about 7.30 P.M. (one male); between 7–10 P.M. three females spawned in quantity two thirds maximum in one dish; three others spawned in quantity in another dish (about 30 chitons per dish); no eggs obtained from dish in running water.

3d night, no eggs obtained; experiment not set until 10 P.M.

Remark.—Spawning distinctly revived. For ten days preceding no eggs obtainable for class work. Was unable to get eggs for embryology class during first half of July. Spawning revived three days before full moon.

VIII.

Experiment 8, July 16–19.

1st night, about 50 chitons placed in crystallizing dish on laboratory table at 10 P.M. (too late). (Collected during afternoon of July 16.) No eggs first night.

2d night, five specimens spawned rather lightly one fourth maximum; one mass of considerable size, three fourths maximum or more; eggs in addition scattered all over the bottom of the dish.

3d night, two or three females spawned lightly before 10 P.M.; very few eggs; one fair-sized group.

4th night, no eggs.

Remark.—Spawning by this group was not particularly heavy. More eggs were spawned by previous group July 15. Not a great difference and not inconsiderable.

* Full moon, July 19.

IX.

Experiment 9, July 20–22.

40 specimens collected July 20. Placed on laboratory table at 6 P.M. Three females spawned lightly before 10 P.M. About 50 eggs each. None after 10 P.M.

2d night, placed on laboratory table at 7 P.M.; three or four spawned one third to one half maximum or more before 10.30 P.M., mostly before 9 P.M.; spermatozoa abundant; none after 11 P.M.

3d night, four females spawned about twenty eggs each; practically none at all.

Remark.—Only fair spawning activity. Plenty of eggs for class, however, and approximately equal to the two preceding experiments, 7 and 8. Spermatozoa were abundant.

X.

Experiment 10, July 25–26.

70 specimens collected Monday, July 25. Mostly half or two thirds grown, but sexually mature.

1st night, five spawned, one half to two thirds maximum.

2d night, six or seven spawned; four of them one half to two thirds maximum; impression of fair or good spawning activity; good quantity as spawning goes this year; perhaps as heavy as the best this season; comparable to that of the last week in June, but probably not quite equal to it.

* Last Quarter, July 27.

XI.

Experiment 11, July 27–29.

75 Chitons collected Wednesday, July 27. Placed in two dishes upon laboratory table. None spawned 1st night, except a very few eggs from two or three individuals. Negligible.

2d night, six specimens spawned about one third to one half maximum; all spawned before 10 P.M.; mostly between 8–10 P.M. (There were only 45 specimens used this 2d night. Several had been lost.)

3d night, no eggs.

Remark.—Good spawning activity.

XII.

Experiment 12, August 1–3.

100 Chitons collected Monday, August 1.

1st night, placed in three dishes on laboratory table; three females spawned very lightly before 10 P.M. about one fifth maximum or less; none after 10 P.M.

2d night, two dishes placed on table; one left in running water; about 75 specimens in all, some having been lost; four females spawned about 25 eggs each by 10 P.M.

3d night, no eggs.

Remark.—Spawning greatly reduced and all but suppressed. Spawning this year much lighter than last year.

XIII.

Experiment 13, August 5–6.

40 Chitons collected Friday, August 5.

1st night, placed in two dishes at 7 P.M.; no eggs or only one half dozen eggs from one female; one male shed spermatozoa before 10 P.M.

2d night, four females spawned about two thirds maximum and a fifth one half maximum; impression of fair spawning activity; good for this year; all done before 11 P.M.

3d night, about 25 eggs from each of three females; practically none; done before 11 P.M.

* New moon, August 3.

XIV.

Experiment 14, August 9–10.

60 Chitons collected Tuesday, August 9, but not brought to laboratory until next day.

2d night, two females spawned about one fourth maximum.

XV.

Experiment 15, August 10–12.

25 Chitons collected August 10—Wednesday.

1st night, none spawned.

2d night, two spawned one fourth maximum; *very light.*
3d night, one female spawned about one sixth maximum.

Remark.—Spawning very light. Almost none.

*First Quarter, August 10.

XVI.

Experiment 16, August 12–14.

45 Chitons collected Friday, August 12. Placed in two dishes.
1st night, none spawned.
2d night, three females spawned (two one fifth maximum; one one half maximum; two in one dish and one in the other); a fourth specimen spawned 50–100 eggs after 10 P.M.
3d night, no eggs.

Remark.—Impression of light spawning activity. Spawning all but suppressed during past week. Quantity of eggs not sufficient for embryological studies.

XVII.

Experiment 17, August 15–17.

100 Chitons collected Monday afternoon. Arrived 5 P.M. Put in dishes 7.30 P.M. Four dishes.
1st night, no eggs.
2d night, abundance of spermatozoa shed; water clouded; also quantities of eggs from three to five females in each dish one third to two thirds maximum.
3d night, August 17, two females spawned one fourth maximum; spermatozoa very abundant, but very few eggs.

Remark.—Very distinct revival of spawning activity; most abundant this year.

*Full moon, August 18.

XVIII.

Experiment 18, August 18–20.

50 large Chitons collected August 18.
1st night, animals remained in aquarium in laboratory (not discovered).

2d night, set in two dishes on laboratory tables; one female of medium size spawned maximum quantity (approximately 3,000 eggs); two others spawned one sixth to one fourth maximum; a fourth laid a few eggs, all before 10 P.M.; an enormous quantity of spermatozoa shed; white cloudy water.

3d night, August 20, no eggs; small amount of spermatozoa.

Remark.—Not many females spawned, but the greatest mass of eggs obtained during this year came from one female of this group. Spermatozoa were particularly abundant. An abundance of spermatozoa has been noted at each full moon, water becoming so turbid that one could not see eggs on the bottom of the shallow dish.

XIX.

Experiment 19, August 22–23.

55 Chitons collected Monday, Aug. 22.

1st night, no eggs.

2d night, only about 50 eggs from a single female; no visible spermatozoa.

Remark.—Practically no spawning.

XX.

Experiment 20, August 24–27.

60 specimens collected Wednesday, August 24.

1st night, no eggs.

2d night, one female spawned two thirds maximum between 8–10 P.M.; one male shed spermatozoa; two other females shed eggs before 11.30 P.M., about 300 and 100, respectively; a fourth female spawned during the night after 11.30 P.M. one half to two thirds maximum.

3d night, two spawned one third maximum before 10 P.M.

Remark.—Fair spawning activity.

* Last Quarter, August 26.

XXI.

Experiment 21, August 26–28.

Chitons collected from both Nobska Light and Vineyard Haven, August 26. About 100 specimens.

1st night, no eggs.

2d night, no eggs up to 10 P.M.; one female spawned 20 eggs after 10 P.M.

3d night, one female spawned 100 eggs.

Remark.—Practically no spawning. Many specimens used. Season rather late. Spawning sporadic.

XXII.

Experiment 22, August 29–30.

60 Chitons collected August 29.

1st night, no eggs.

2d night, six females spawned very lightly one fifth to one tenth maximum; only a few eggs each; several males shed spermatozoa.

Remark.—Represents a distinct increase over preceding nights. None after 10.30 P.M. Spawning per individual pretty light, however.

XXIII.

Experiment 23, August 31–September 1.

120 Chitons collected August 31. Placed in three dishes.

1st night, no eggs.

2d night, nine females spawned; three one half maximum, others one fourth to one fifth maximum; very considerable spawning and much spermatozoa in one dish; two dishes with little spermatozoa.

3d night, no eggs.

Remark.—Good spawning activity for time of season.

* New moon, September 1.

XXIV.

Experiment 24, September 6–7.

85 Chitons collected Tuesday, September 6. Placed in three dishes.

1st night, no eggs.

2d night, two females spawned in one dish two thirds maximum; one spawned in another dish one fourth maximum, 8–10.30 P.M.; third dish, no eggs.

Remark.—Experiments for the season closed September 7. For some days there had been indications that the spawning season was nearing its end. Impression of fair spawning activity, although from only a few individuals. The experiments show that heavy spawning begins within a few days of full moon and continues to third quarter, or a few days after third quarter. The lightest spawning occurs between new moon and full moon, especially pronounced around first quarter.

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